Thesis for doctoral degree (PhD)

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Development of an Efficient Test for Autoimmune disease using Gold Nanoparticles

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Anantdeep Kaur declare that this thesis, is submitted in fulfilment of the requirements for the award of PhD degree, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution. This research is supported by an Australian Government Research Training Program Scholarship.

Anantdeep Kaur

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List of patents and papers

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Kaur, A., Shimoni, O. & Wallach, M. 2017, 'Coeliac disease: from etiological factors to evolving diagnostic approaches', Journal of Gastroenterology, vol. 52 (9), pp. 1001–1012. https://doi.org/10.1007/s00535-017-1357-7*.

Kaur, A., Wallach, M. & Shimoni, O. 2018, 'A novel diagnostic test for coeliac disease using gliadin coated gold nanoparticles' (under review).

Kaur, A., Shimoni, O. & Wallach, M. 2018, 'A novel screening test for coeliac disease using peptide functionalised gold nanoparticles', World Journal of Gastroenterology, vol. 24(47), pp. 5379-5390*.

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Statement of contribution of authors

Paper I: Coeliac disease: from etiological factors to evolving diagnostic approaches

Author contributions: I (Anantdeep Kaur) researched and reviewed the literature and analysed data. I (Anantdeep Kaur) wrote the paper with critical revisions related to the intellectual content of the manuscript from Prof Michael Wallach and Dr Olga Shimoni.

Paper II: Novel diagnostic assay for coeliac disease using gliadin coated gold nanoparticles

Author contributions: Dr Olga Shimoni and Prof Michael Wallach designed research, I (Anantdeep Kaur) performed research and analysed data. I (Anantdeep Kaur) wrote the paper with critical revisions related to the intellectual content of the manuscript from Prof Michael Wallach and Dr Olga Shimoni.

Paper III: A novel screening test for coeliac disease using peptide functionalised gold nanoparticles

Author contributions: Dr Olga Shimoni and Prof Michael Wallach designed research, I (Anantdeep Kaur) performed research and analysed data. I (Anantdeep Kaur) wrote the paper with critical revisions related to the intellectual content of the manuscript from Prof Michael Wallach and Dr Olga Shimoni.

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3MT Topic: 'To go gluten free or not?'

University of Technology Sydney

Table of Contents

List of Tables and Figures

List of Abbreviations

CD	Coeliac Disease
AGA	Anti-gliadin antibody
RCD	Refractory Coeliac Disease
HLA	Human Leukocyte Antigen
MHC	Major Histocompatibilty Complex
TG2	Tissue Transglutaminase
FADD	Fas associated via death domain
DED	Death Effector Domain
IL	Interleukin
NK	Natural killer
TCR	T cell receptor
LMW	Low molecular weight
CDR	Complementarity determining region
DGP	Deamidated gliadin peptides
Ig	Immunoglobulin
Anti-EMA	Anti-endomysial antibodies
Anti-tTG	Anti-transglutaminase antibodies
Anti-DGP	Anti-Deamidated gliadin peptide
MMR	Mumps and Rubella vaccine
HAV	Hepatitis A Virus
EGF	Epidermal Growth Factor
NCGS	Non Coeliac Gluten Sensitivity
IFA	Indirect immunofluorescence

RIA	Radioimmunoassay
GFD	Gluten free diet
SPR	Surface plasmon resonance
DIG	Diffusion in gel
IBD	Inflammatory bowel disease
GIP	Gluten immunogenic peptides
AuNP	Gold Nanoparticle
LSPR	Localised Surface Plasmon Resonance
PEG	Polyethylene glycol
ELISA	Enzyme linked immunosorbent assay
HCG	Human Chorionic Gonadotrophin
Mab	Monoclonal antibody
CTAB	Cetyl trimethylammonium bromide
IPA	Isopropyl Alcohol
BSA	Bovine Serum Albumin
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
PBS	Phosphate Buffer Saline
DLS	Dynamic Light Scattering
℃	Celsius
Å	Angstorm
kDa	kiloDalton

ABSTRACT

Coeliac disease results because of an unusual immune response to the digestion of gliadin protein. Patients suffering from the disease show varying degrees of chronic inflammation within the small intestine ranging from mild lesions to completely flat mucosa. The immune response in turn, leads to the production of a number of cytokines and antibodies that are linked to the disease and its pathological effects. The released antibodies can be used as specific biomarkers for developing reliable diagnostic tests. Over the years, different approaches like the use of mucosal biopsy, genetic typing of the disease associated gene, gliadin induced cytokines, antibodies and auto-antibodies have been used to develop diagnostic tests for coeliac disease. However, in spite of the encouraging initial results in terms of sensitivity and specificity, the existing tests have limited scope as point-of-care tests.

The current study was aimed at developing a coeliac diagnostic assay based on the properties of the gold nanoparticles combined with the specificity of the antibodies from serum as well as saliva. In this study, I developed a novel diagnostic test for coeliac disease based on the coating of gold nanoparticles with gliadin, the highly antigenic protein responsible for inducing the symptoms of coeliac disease. A novel protocol for binding the hydrophobic gliadin protein on the surface of the gold nanoparticles was developed in this study. This was followed by the development of a simple, single antibody serology based diagnostic test.

Finally, I used the assay on thirty patient serum samples in a blinded assessment and compared the results with the data from previously run serological and pathological tests on these patients. When tested on real patient samples, the data showed that the developed assay had an overall accuracy of over 96%.

Furthermore, I developed an assay based on the coating of gold nanoparticles with a peptide sequence derived from gliadin. To develop the serological assay based on the peptide functionalised nanoparticles, I first established a stable suspension of peptide coated gold nanoparticles and then tested the assay on spiked serum samples. I then used the assay to test thirty patient serum samples and found that the peptide functionalised nanoparticle-based assay could distinguish coeliac disease from non-coeliac disease patients with an accuracy of 86.6%.

This study demonstrates the potential of gold nanoparticle-based approach to be adapted for developing a point-of-care screening assay for diagnosis for CD. The developed assay could be a part of an exclusion based diagnostic strategy and prove beneficial for testing high coeliac disease risk populations.